Clinical manifestations including the EULAR Sjögren's Syndrome Disease Activity Index in anti-Ro52/SS-A antibody-seropositive patients with Sjögren's syndrome

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Abstract

Background: Relationship between anti-Ro52/SS-A antibody (anti-Ro52) and clinical manifestation of Sjögren's syndrome (SS) was sporadically described regarding involvement of internal organs.

Objectives: To determine the clinical factors relevant to anti-Ro52 with SS.

Methods: We conducted a retrospective study of subjects with suspected SS (n=149), patients with rheumatoid arthritis (RA) (n=62), and healthy subjects (n=50). We analyzed components of the American-European Consensus Group (AECG) criteria, Raynaud's phenomenon (RP), anti-centromere antibody (ACA), serum IgG, rheumatoid factor, and the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI).

Results: Among the 149 suspected SS subjects, 115 subjects were classified as having SS. Anti-Ro52 was observed in 70 SS patients (60.9%), of whom 31 patients had markedly elevated anti-Ro52 (>500 U/ml). In the SS group, three patients with anti-Ro60−/anti-Ro52+ and 17 patients without anti-Ro were observed. Compared to ACA, the relevance of positive anti-Ro52 toward positive anti-Ro60 was significantly higher ($p<0.05$). We observed relevance between the anti-Ro52 concentration and anti-Ro60 that was significantly affected by xerophthalmia, xerostomia, ACA seropositivity, RP, serum IgG level, and RF. The anti-Ro52 concentration well discriminated six clinical factors (ROC AUC >0.75) for ACA seropositivity, ESSDAI score ≥1, and RF or moderately high serum IgG, focus score ≥1, and anti-La/SS-B antibody seropositivity (ROC AUC >0.7). A linear relationship between the ESSDAI score and anti-Ro52 was observed.

Conclusion: Significant relevance between anti-Ro52 and anti-Ro60 as well as significant items including the ESSDAI regarding the anti-Ro52 concentration was revealed. Regarding relevance toward anti-Ro60, anti-Ro52 had a higher association than ACA.

(246/250 words)

Introduction

Sjögren's syndrome (SS) is a systemic autoimmune disease that has unique clinical manifestations including xerostomia, xerophthalmia, and extraglandular clinical manifestations such as interstitial pneumonia and tubulointerstitial nephritis [1, 2]. SS also has characteristic autoantibodies including anti-Ro/SS-A and La/SS-B autoantibodies [3]. Although anti-Ro/SS-A antibody (anti-Ro) is not specific for SS (it is also detected in sera from patients with systemic lupus erythematosus [SLE]), Ro/SS-A antigen has two distinct subtypes: Ro52 (which was identified as TRIM21) and Ro60 [4]. Regarding the clinical significance of anti-Ro60/SS-A antibody (anti-Ro60) which usually co-exists with anti-Ro52/SS-A antibody (anti-Ro52), the existence of anti-Ro/SS-A antibody is a critical item in the 2002 American-European Consensus Group (AECG) [5] classification criteria, the 2016 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria [6], and the 1999 revised Japanese Ministry of Health criteria [7] for primary SS.

Regarding the association between the titer of anti-Ro52 and clinical activity in patients with SS, there is a single report (by the Sjögren's Syndrome Research Group, in a Spanish cohort [8]) that showed that age, the presence of Raynaud's phenomenon (RP), the serum IgG level, and the presence of anti-La/SS-B antibody were associated with the titer of anti-Ro52. There has been no subsequent publication with respect to the anti-Ro52 titer and clinical manifestations. In particular, there has been no report establishing the relationship between the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) and anti-Ro52. A French study reported that anti-Ro52 was closely associated with interstitial lung disease [9], but the relationship between clinical manifestations and anti-Ro52 in Japanese patients with SS has not been determined. We conducted the present study to investigate this relationship in a Japanese SS patient series, and as an additional analytical item, we focused on the anti-centromere antibody (ACA)-seropositive SS subgroup (ACA-positive SS), which accounts for approx. 10% of patients with SS. As it was reported that ACA-positive SS patients had low percentages of anti-Ro, the subgroup of ACA-positive SS patients is recognized as a distinct group that has a high prevalence of RP and normal serum IgG levels [10, 11]. Here, to determine the clinical factors relevant to anti-Ro52/SS-A antibodies in patients with SS, we retrospectively analyzed cases of patients with SS, patients with rheumatoid arthritis (RA), and healthy controls. The results of our analyses demonstrate the seroprevalence of ACA and relevant clinical manifestations including the ESSDAI score of anti-Ro52/SS-A antibody-seropositive SS patients.

Subjects And Methods

Kits and reagents

Anti-Ro (Ro60) and anti-La/SS-B antibody were detected by the Mesacup SS-A/Ro test and SS-B/La Test, respectively (Medical & Biological Laboratories, Nagoya, Japan). The enzyme-linked immunosorbent assay (ELISA) was used for in Mesacup SS-A/Ro and SS-B/La tests. Briefly, immobilized native antigens including SS-A/Ro and SS-B/La were subjected to antigen-antibody reaction. The 101-fold diluted single samples, positive control as positive human serum, negative control as normal human serum were used. After washing, the reaction was incubated with peroxidase-conjugated anti-human immunoglobulin polyclonal goat antibody as a second as secondary reaction followed by measurement of A_{450} absorbance. Positive thresholds of Mesacup SS-A/Ro were determined in 930 patients with connective tissue disease by comparing with results determined by double immunodiffusion methods, in which positive zone was determined by highest index with respect to sensitivity and specificity. ACA was quantified by an ELISA kit (Mesacup-2 test CENP-B; Medical & Biological Laboratories). Anti-Ro52 was measured by EliA Anti-Ro52 (ThermoFisher Scientific, Waltham, MA, USA); briefly, this product is based on FEIA (fluorescence enzyme immunoassay) technology and uses genetically produced recombinant Ro 52-kD protein on the solid phase. Anti-SS-A/Ro52 antibody was also measured by ELISA-based method. Briefly, human recombinant SS-A/Ro (Ro52) protein coated the EliA Ro52 wells was used for antigen-antibody reaction. The 100-fold diluted single samples, positive control as positive human origin EliA ANA positive control, negative control as EliA IgG/IgM/IgM were used. After washing, the reaction was incubated with b-Galactosidase anti-human immunoglobulin monoclonal mouse antibody as a second

as secondary reaction followed by measurement of A_{445} absorbance. Positive thresholds of anti-SS-A/Ro52 antibody were determined as follows; Samples from 400 blood donors including the patients with SS and the relevant disease control were used by calculating the 99 and 98 percentile. After receiver operating characteristic (ROC) curve was calculated, the cut-off point was defined by determining highest sensitivity and specificity.

Subjects

We enrolled 149 subjects suspected of having SS who visited Nagasaki University Hospital between 2009 and 2018 **(Fig. 1)**. The classification of primary (p) SS was determined based on the 2002 AECG classification criteria [5]. Among the 149 subjects with suspected SS, 115 subjects were classified as having SS based on the AECG criteria **(Fig. 1)**. The 115 patients with SS were classified as 87 primary SS and 28 secondary SS. There were 28 patients with secondary SS; these cases were complicated with RA in 13 patients, with SLE (n=6), mixed connective tissue disease (n=5), systemic sclerosis (SSc; n=2), polymyositis (PM; n=1) and anti-phospholipid syndrome (n=1). One PM patient had neither anti-Ro52 nor anti-Jo-1 antibody. The degree of mononuclear cell infiltration in the subjects' labial salivary glands (LSGs) was evaluated based on the Chisholm & Mason grading or a focus score (FS) determined by Greenspan [12, 13]. We retrospectively evaluated the disease activity of SS by using the ESSDAI [14, 15], which is composed of 12 items used to determine the systemic involvement of SS. The present FS calculation was subjected to the standardization method recommended by the EULAR SS Study Group [16].

As a disease control group, we examined the cases of 62 patients with rheumatoid arthritis (RA) that had been classified based on the 2010 ACR/EULAR classification criteria or the 1987 American Rheumatism Association criteria for RA [17, 18]. For the exclusion of latent SS, we excluded RA patients with sicca symptoms including xerostomia and/or xerophthalmia and anti-Ro60 that was determined by the Mesacup SS-A/Ro test and SS-B/La Test. Blood samples from 50 healthy subjects who showed participation intention were used as normal controls. Healthy subjects were tested only for anti-Ro52 by EliA Anti-Ro52.

For the calculation of the focus score (FS), images of salivary glands were captured by a microscope (BZ-X-710, Keyence, Osaka, Japan), and whole areas of salivary glands were measured by the hybrid cell count system, then FS was manually calculated as the number of foci per $4mm^2$ according to the method described by Fisher et al [16]. This case-control study involving patients' medical records and sera was performed with the disclosure of information according to the approval of the Clinical Studies Ethics Committee of Nagasaki University Hospital (approval no. 18121007). Handling blood samples from healthy subject was approved by this Committee because this opt-out disclosure operation is based on ethical guidelines for medical and health research involving human subject determined in Japan.

Statistical analysis

For comparison of age and sex among the patients with SS, the patients with RA and healthy subjects, Wilcoxon rank-sum test and Fisher exact test were used, respectively. We included the following items in the analyses as clinical factors of interest: AECG criteria components including xerostomia, xerophthalmia, salivary secretion by Saxon test, lacrimal secretion by Schirmer's test, the focus score (FS), labial salivary gland biopsy (LSGB) grading (0–2/3/4), anti-Ro/SS-A antibody, anti-La/SS-B antibody, and relevant items including RP, ACA, serum IgG, rheumatoid factor (RF), and the ESSDAI score.

The concentration of anti-Ro52 and anti-Ro60 was transformed to logarithm with base 10. The association of clinical factors with relevance between anti-Ro52 and anti-Ro60 was inferred as the 95% confidence interval (95%CI) of the regression coefficient of a term of interaction between anti-Ro52 and the clinical factor in a linear regression model, which regressed anti-Ro60 onto the interaction term with covariates of anti-Ro52 and the clinical factor. The model did not include other covariates to control for confounders. The regression coefficients were estimated by MM-estimator with bisquare psy-function [19, 20] (for all of the linear regression analyses). We used the multiple imputations by chained equation (MICE) method [21] to estimate the regression coefficients. The patterns of missingness are shown in Supplemental Figure S1 (labeled "NA" on the x-axis) and Supplemental Figure S2 (the right column of each plot). The contour of two-dimensional probability density was drawn based on a kernel density estimate with the Gaussian kernel with bandwidth selected by the "solve-the equation" estimator [22, 23]. The linear relationship between the ESSDAI score and the levels of antibodies against the two respective subtypes of Ro/SS-A antigen were analyzed by MM-estimator with bisquare psy-function.

We compared the odds of anti-Ro60 positivity between the positivity of ACA and that of anti-Ro52 by using the ratio of the two odds ratios (the ratio of ORs). The null hypothesis that the ratio is 1 was tested via a permutation test of anti-Ro60 positivity. We assessed the associations between markedly elevated anti-Ro52 (>500 U/ml) and each of the clinical manifestation by determining the OR, and the statistical significance of each association was tested by Fisher's exact test. We evaluated the associations between respective clinical factors and the concentration of antibodies against the two respective subtypes of Ro/SS-A antigen by determining the maximal information coefficients [24]. The null hypothesis of independence between respective clinical factors and the concentration was tested by a permutation test. The odds of positivity in respective characteristics given the concentration ~~titer~~ of anti-Ro52 were evaluated with the area under curve (AUC) of a receiver operator characteristic (ROC) curve [25]. The confidence intervals of sensitivity and specificity were obtained from 2,000 bootstrap samplings. Hypothesis testing was conducted with the significance level of 0.05 without adjustment for multiple comparisons. All statistical analyses were conducted under the R environment [26] ver. 3.6.0, especially the *robustbase* package [27] ver. 0.95-5 in the robust regression, the *mice* package [21] ver. 3.5.0 in the multiple imputation, and *pROC* package [28] ver. 1.15.3.

Results

The seroprevalence of anti-Ro52 in the SS patients

The 115 patients with SS were classified as 87 primary SS and 28 secondary SS. Among these 115 patients with SS, 70 patients (60.9%) were anti-Ro52 positive **(Fig. 2A)**. The p value for group differences of age between the patients with SS vs healthy subjects and the patients with SS vs the patients with RA was <0.001 and 0.018, respectively. In addition, the p value for group differences of sex between the patients with SS vs healthy subjects and the patients with SS vs the patients with RA was <0.001 and 0.025, respectively. Therefore, no significant differences in the distribution of age and sex were observed among these 3 groups. There were two cases (3.2%) of positive anti-Ro52 among the 62 patients with RA, and there was no positive case among the 50 normal control subjects **(Fig. 2A)**. The 2 secondary SS patients with ACA+SSc patients fulfilled 2013 ACR/EULAR classification criteria for SSc and had neither anti-Ro52 nor anti-Ro60. The group of 115 SS patients included 95 patients with anti-Ro60 (82.6%). There were 34 non-SS subjects who were not classified as SS by AECG criteria. In the non-SS subjects, 7 subjects were positive for anti-Ro52 **(Fig. 2A)**.

The background characteristics of the patients with SS according to the presence or absence of anti-Ro52 are summarized **(Table 1)**. Five items were significantly high in the SS patients with anti-Ro52. We also examined the subjects' treatment for sicca symptoms and the use of immunosuppressants. There was no case of congenital heart block (CHB) in the patients' medical records. According to their status as being with or without anti-Ro60, the SS patients with anti-Ro52 were divided 2 groups. Anti-Ro60+ SS patients included anti-Ro60+anti-Ro52+ patients (n=67); and the anti-Ro60+anti-Ro52− patients (n=28). In contrast, anti-Ro60- SS patients included the anti-Ro60-anti-Ro52+ patients (n=3), and the anti-Ro60-anti-Ro52− patients (n=17) **(Fig. 2B)**. We also observed markedly high anti-Ro52 (≥500 U/ml) in 31 (44.3%) of the 70 anti-Ro52-positive patients with SS **(Fig. 2C)**.

Table 1. Clinical characteristics of the SS patients

Variables at diagnosis	Anti-Ro52 positive (n=70)	Anti-Ro52 negative (n=45)	p-value
Age, yrs; mean±SD	58.8±14.6	58.8±14.0	0.92
Female patients, n (%)	68 (97.1)	41 (91.1)	0.16
Xerophthalmia, n (%)	42 (60.0)	33 (75.0)	0.07
Xerostomia, n (%)	53 (75.7)	39 (86.7)	0.12
Positive Saxon test, n (%)	54 (84.4)	32 (74.4)	0.15
Positive Schirmer's test, n (%)	41 (68.3)	30 (75.0)	0.31
Anti-Ro60, n (%)	67 (95.7)	28 (62.2)	<0.01*
Anti-La/SS-B antibody, n (%)	31 (44.3)	8 (17.8)	<0.01*
Positive ANA, n (%)	67 (95.7)	41 (91.1)	0.27
Positive RF, n (%)	50 (75.8)	12 (29.2)	<0.01*
Serum IgG mg/dl, mean±SD	1996 ± 619	1579 ± 461	<0.01*
WBC count, $x10^3/\mu l$	5018 ± 2434	5055 ± 1412	0.92
Hemoglobin, g/dl	12.3 ± 1.5	12.7 ± 1.1	0.04*
Platelet count, $x10^3/\mu l$	20.3 ± 8.6	22.5 ± 5.1	0.08
Focus score, mean±SD	4.5 ± 2.8	4.3 ± 3.6	0.78
ESSDAI score, mean±SD	2.0 ± 2.0	0.8 ± 2.1	<0.01*
Use of ophthalmic solutions, n (%)	18 (25.7)	11 (24.4)	0.53
Use of muscarinic receptor agonists, n (%)	25 (35.7)	14 (31.1)	0.38
Use of immunosuppressants, n (%)	15 (21.4)	4 (8.9)	0.06

*p<0.05. Welch's t-test or Fisher's exact test was used. Ophthalmic solutions included chondron instillation, purified sodium hyaluronate, artificial tear ophthalmic solution, rebamipide ophthalmic suspension, and diquafosol sodium. Muscarinic receptor agonists included cevimeline hydrochloride hydrate and pilocarpine hydrochloride. Immunosuppressants included oral prednisolone (PSL),

tacrolimus, and mizoribine. PSL was used for 9 of the 15 anti-Ro52-positive secondary SS cases and 3 of the 4 anti-Ro52-negative secondary SS cases. ANA: anti-nuclear antibody, anti-Ro52: anti-Ro52/SS-A antibody, anti-Ro60: anti-Ro60/SS-A antibody, ESSDAI; EULAR Sjögren's Syndrome Disease Activity Index, RF: rheumatoid factor. WBC: white blood cell.

Although we identified anti-Ro52 positivity in 75% (63/84) of the anti-Ro60+ACA− patients with SS, there was only one anti-Ro52-positive case among the 16 anti-Ro60-ACA+ patients with SS **(Fig. 2D)**; this case showed an equivocal anti-Ro52 concentration (7.44 U/ml) and was considered anti-Ro52-positive in this study. The odds ratio of anti-Ro60 positivity over anti-Ro52 positivity was 13.2 (95%CI: 3.4–76.0). The odds of anti-Ro60 being positive were significantly higher in the anti-Ro52-positive patients than in the ACA-positive patients ($p<0.05$, permutation test for ratio of odds ratio).

Clinical characteristics associated with relevance between anti-Ro60/Ro52

As described above, our present findings replicate the association between the positivity of anti-Ro52 and that of anti-Ro60 that had been reported previously [29, 30]. We also determined the clinical characteristics with respect to the increment of anti-Ro60 concentration toward increment of the anti-Ro52 concentration. The results of our analyses revealed that the effect of the following clinical characteristics were significant (the values in parentheses are 95%CIs of the regression coefficients of interaction terms): xerophthalmia positivity (-9.49 to -0.42), xerostomia positivity (-8.23 to -3.15), ACA positivity (-7.85 to -1.80), RP positivity (-7.45 to -1.27), high serum IgG (0.02 to 0.85), and RF positivity (1.43 to 12.24).

The relationships between the levels of the two subtypes of anti-Ro are illustrated **(Figure 3)**. The subjects were stratified by the positivity of the respective clinical characteristics, and each subject subgroup's data were plotted separately. The figure's scatterplots reveal a blank range for anti-Ro60 (the boundaries are 4.4 and 34.9 on the x-axis) which divided the subjects into two parts (the lower-left part and the right side in the plots). The unbalanced nature of the density of values in the lower-left parts of the scatterplots among the subgroups is clear, especially for the four items in which significant downward effects of positivity on the regression coefficient were revealed; e.g., between xerophthalmia (−) and xerophthalmia (+). It is possible that the significance of the regression coefficients of the interaction terms is attributable to this clustered unbalancedness. As we noted above, it was also clear that the relationships between the levels of anti-Ro52 and anti-Ro60 were not linear, but we used a linear regression in order to grasp the characteristics of the global relationship.

The association between clinical characteristics and anti-Ro60/Ro52

We compared the concentration of anti-Ro52 and anti-Ro60 between the subgroups of subjects classified by components in the AECG criteria and by other items including RP, ACA, RF, and serum IgG **(Suppl. Fig. S1)**. The comparison revealed that three characteristics (RP, ACA, and IgG) with which subgroups were created were significantly different according to the titer for both antibodies ($p<0.05$ for each characteristic). Regarding the anti-Ro52 concentration, the differences were significant in three additional

characteristics: xerophthalmia, anti-La/SS-B antibody, and RF. For the anti-Ro60 concentration, there was no additional characteristic with which the association was significant aside from RP, ACA, and IgG.

We determined the maximal information coefficients to identify the clinical characteristics with significant dependency between anti-Ro52 or anti-Ro60 or both, and the results demonstrated that the dependency between anti-La/SS-B and both anti-Ro52 and anti-Ro60 was significant ($p<0.05$ for each antibody) **(Suppl. Fig. S2)**. We also investigated which clinical characteristics are associated with the anti-Ro52 concentration by determining the ROC curve **(Fig. 4)**. The characteristics in which positivity was highly discriminated by the concentration of anti-Ro52 were as follows: ACA, ESSDAI ≥1, and RF (AUC >0.75, respectively) **(Fig. 4)**. Moderately discriminated characteristics were serum IgG, FS ≥1, and anti-La/SS-B antibody (AUC >0.70, respectively) **(Fig. 4)**.

We analyzed the linear relationships between the ESSDAI score and levels of antibodies against the two respective subtypes of Ro/SSA antigens. The range of our subjects' ESSDAI scores was 0 to 17. In the linear regression, the patients with the ESSDAI score >4 were put together in the subgroup of 'ESSDAI ≥4,' and in this analysis, significant linearity between the ESSDAI scores and anti-Ro52 was confirmed **(Fig. 5)** as anticipated from the correlation with anti-Ro60. The details of the ESSDAI scores of the entire group of SS patients and of the anti-Ro52-positive SS patients are also shown **(Suppl. Fig. S3)**, indicating the presence of a high frequency of the biological item and a low frequency of the articular item. We also displayed the level of anti-Ro52 and anti-Ro60 according to positive ESSDAI domain **(Suppl. Fig. S4 and S5)**, indicating that the level of anti-Ro52 in all domains that had positive number showed more than cutoff value, 10 U/ml.

To determine the influence of secondary SS, we examined the clinical characteristics that were relevant to the association between the anti-Ro52 and anti-Ro60 concentration in primary SS by deleting the data of the 28 patients with secondary SS, and we obtained the following significant findings: xerostomia positivity (-8.24 to -3.19), ACA positivity (-7.84 to -1.77), RP positivity (-7.48 to -1.18), high serum IgG (0.04 to 0.86), RF positivity (1.34 to12.10) (the values in the parentheses are 95%CIs of the regression coefficients of interaction terms). Compared to the findings of total SS in Figure 3, the significance of xerophthalmia (−9.56 to 0.90) disappeared **(Suppl. Fig. S6)**.

Discussion

The results of our retrospective analyses demonstrated that anti-Ro52 was more frequently detected in the sera from patients with SS compared to the sera from RA patients and normal subjects. There were 7 patients with anti-Ro52 among 34 non-SS subjects. Among these 7 patients, 5 subjects were positive for anti-Ro60 and 2 subjects were positive for ACA without salivary gland biopsy. The reasons why these 7 subjects were not classified as SS by AECG criteria might be coexistence of anti-Ro antibodies and lack of information for pathological findings. In terms of relevance toward positive anti-Ro60, the odds of anti-Ro52 positivity were significantly high compared to anti-Ro52 and ACA. In a simple comparison without a test of the relevance between anti-Ro52 and anti-60, we observed that the group of SS patients who were

anti-Ro52-positive had significantly high prevalences of anti-Ro60, anti-La/SS-B antibody, and RF, high serum IgG levels, and high ESSDAI scores. The extent of the increase in the level of anti-Ro52 for the extent in the level of anti-Ro60 was significantly higher in the subjects with high serum IgG and high RF. Contrary to these two characteristics, the extent was significantly lower in the subjects with the following items compared to those without them: xerophthalmia, xerostomia, ACA, and the presence of RP. Our results also identified six clinical parameters (including the ESSDAI score) that were significantly associated with the anti-Ro52 level.

Although Ro52 was initially reported as a component of SS-A particle, it is structurally distinct from Ro60. Ro52 has four structural domains: RING, B-box, coiled coil (CC), and B30.2/PRYSPRY regions [31]. Because motifs that have three regions including RING, B-box, and CC are describes as tripartite motif proteins (TRIMs), Ro52 is also described as TRIM21 [4, 32]. With regard to the function of Ro52, the ubiquitination of intranuclear target molecules as well as E3 ligase activity are known [33]. Type 1 interferon is known to induce the translocation of Ro52 to the nucleus [34]. A relationship between the above molecular mechanisms and the clinical manifestations identified in the present study (including high serum IgG level and ESSDAI score) has not yet been determined, but the ubiquitination of Ro52 antigen or a change in cellular distribution might be associated with anti-Ro52-positive patients with SS. Since it is possible that the change of ubiquitination by anti-Ro52 may increase inflammation in each organ in patients with SS, the serum IgG level, RF, FS, and ESSDAI score (which showed an AUC >0.7 with respect to anti-Ro52) might be explained by the effect of anti-Ro52 on ubiquitination. In addition, this biochemical action of anti-Ro52 and the wide distribution of Ro52 antigen in various organs [35, 36] might be related to the relevance between ESSDAI among the items with an AUC >0.7 and the change of anti-Ro52 concentration.

Clinically, anti-Ro52 is found in SS as well as other autoimmune diseases [37, 38]. Although the specific biochemical reasons are not known, a high frequency of the co-expression of anti-Ro52 with anti-Jo-1 antibody was reported in patients with inflammatory myopathy, although there was no difference regarding Ro52 epitope recognition in the presence or absence of anti-Jo-1 antibody [39]. In addition, organ specificity of anti-Ro52 was repeatedly reported in patients with interstitial lung diseases [40] or CHB [41]. In contrast, there are some controversial data from patients with SLE or SSc [42], in which isolated anti-Ro52 had no significance to estimate the clinical activity of these autoimmune diseases; rather, those data indicated that comorbid autoantibodies had higher diagnostic value than anti-Ro52, as noted in a review [43].

Regarding the relevance between the anti-Ro concentration and clinical manifestations in SS, there are few reports (and no reports of a large study). In a 2006 study of pregnancies presenting a risk of CHB, decreased levels of anti-Ro52 IgG1 and IgG4 were observed [44]. It was recently reported that the concentration of anti-Ro, anti-Ro52, and anti-Ro60 were independent factors for fetal CHB [41]. The anti-Ro concentration was observed to fluctuate in SS patients with skin vasculitis, suggesting that a fluctuating concentration ~~titer~~ of anti-Ro was associated with the disease activity of SS, although that study reported only two skin vasculitis cases among 15 patients with SS [45]. In our present analyses, the

anti-Ro52 concentration showed moderate (AUC >0.70) or high (AUC >0.75) discriminability for six clinical factors. The significant linear relationship between the ESSDAI scores and anti-Ro52 detected by the linear regression model in Figure 5 indicates that the anti-Ro52 level was relevant to clinical parameters including the disease activity in SS.

A user's guide to the ESSDAI [14] was released to precisely assess the clinical activity of SS. Ramos-Casals et al. examined a cohort of 921 Spanish patients with SS, and they reported that the most frequently involved organs were the joints, skin, and peripheral nerves [46]. Although a direct correlation between anti-Ro52 and ESSDAI score was not noted in that study, the reason why the details of our patients' ESSDAI scores **(Suppl. Fig. S3)** differed from the findings reported by Ramos-Casals et al. may be because our ESSDAI results revealed a low frequency of articular involvement and no peripheral nerve symptoms. In this report, the total ESSDAI score was lower than that in the SS Group of the Autoimmune Disease Study Group (GEAS) registry [46] in Spanish centers. In the registry, frequency of glandular and articular domains was 34% and 56% that was much higher than frequency in Suppl. Fig. S3. Since we estimate that low frequency of specific domains might influence on low ESSDAI score in Japanese patients with SS, ethnic differences should be taken into account since components of the ESSDAI might not be the same among different geographic regions. Our recent data supported that the frequency of specific domains in ESSDAI was certainly low according to ethnicity [47]. We observed non-ESSDAI cardiovascular items including 14.8% (17/115) of Raynaud’s phenomenon that was reported recently [48] and 1 case of pulmonary arterial hypertension. In addition, 3 cases of autonomic dystonia were observed in 115 patients with SS. Because presence of these clinical manifestations might influence on the frequency of items in ESSDAI, we should carefully observe extra glandular manifestations considering regional characteristics.

Regarding ACA, Earnshaw et al. identified centromere proteins (CENPs) including CENP-A, B, and C [49], which was followed by the molecular cloning of CENP-B [50]. Although CENP-A and C were shown to be involved in the assembly of the kinetochore [51], the function of CENP-B remains unknown. Because CENP-B can bind the specific CENP-B box [52], it was predicted that CENP-B regulates the dynamic state of heterochromatin in centromeres. In a Spanish cohort, anti-Ro52 was found in 35.6% of the patients with SSc, and the patients with anti-Ro52 had a high prevalence of ACA (61.9%) [53]. Regarding the coexistence of ACA and anti-Ro52 in SSc, the Canadian Scleroderma Research Group (CSRG) observed that CENP-B was present in approx. 43% of 194 patients with anti-Ro52-positive SSc, although a biochemical relationship between anti-Ro52 and ACA was not demonstrated [54]. In contrast, we observed significantly low odds of anti-Ro52 seropositivity in our ACA+ SS patients. This paradoxical result suggests a potential difference in the coexistence of anti-Ro52 in ACA+ between SS and SSc. Concerning the differential diagnosis between ACA+ SS and ACA+ SSc, the potential future development of SSc during the course of ACA+SS is an important issue. Given the descriptions that anti-Ro52+SSc had a high prevalence of ACA, the low prevalence of anti-Ro52 in ACA+ SS patients can be helpful to distinguish these two disorders.

Our study has some limitations. We did not use the 2016 ACR/EULAR criteria because the ocular staining test, which is one of the main items in 2016 ACR/EULAR criteria, was not performed in many of our patients. In addition, the use of the Saxon test (one of the stimulated salivary secretion tests used instead of an unstimulated salivary secretion test) might influence the classification of SS. When a sufficient number of enrolled subjects with a complete data set for statistical analyses is obtained, an analysis that uses the 2016 ACR/EULAR criteria will be necessary. Our present findings confirmed the linearity between the levels of antibodies against two respective subtypes of Ro/SSA antigen and the ESSDAI scores (0, 1, 2, 3 and >4); however, as shown in Supplemental Figure S3 (A), this relationship remains unclear in the subjects with ESSDAI scores >4. In addition, clustering tendency of anti-Ro60 determined by Mesacup SS-A/Ro with blank range was not determined biochemically.

Conclusion

Taken together, our results revealed a discriminative expression pattern of anti-Ro52 in ACA+ patients with SS as well as the prevalence of concurrent anti-Ro52 in patients with anti-Ro60+ SS, as already reported. Because these observations differ from known data observed in SSc, these might be new characteristics with regard to the autoantibody profile in SS. Our analyses also revealed that six items are associated with the relevance between anti-Ro52 and anti-Ro60, and six other items are associated with the anti-Ro52 concentration. The influence of anti-Ro52 that was related to the inhibition of ubiquitination and subsequent inflammation toward chronic inflammatory parameters and the ESSDAI might explain in part the significance of these findings. Of course, we should note that the clinical importance of anti-Ro52 was reported in the presence of other autoantibodies such as anti-Ro60. Concerning the findings that showed close association between anti-Ro52 concentration and SS-related clinical items including ESSDAI, anti-Ro52 should be tested to decide therapeutic strategies in daily practice. Large-scale studies of other populations are also necessary, since the present study was of Asian subjects. The relevant clinical manifestations of anti-Ro52 will be clarified in a more global study.

Abbreviations

ACA: anti-centromere antibody, AECG: American-European Consensus Group, CENP: centromere protein, CHB: congenital heart block, ELISA: enzyme-linked immunosorbent assay, ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index, EULAR: European League Against Rheumatism, FS: focus score, LSG: labial salivary gland, LSGB: labial salivary gland biopsy, PSL: prednisolone, pSS: primary Sjögren's syndrome, RA: rheumatoid arthritis, RP: Raynaud's phenomenon, SLE: systemic lupus erythematosus, SSc: systemic sclerosis, TRIM: tripartile motif protein

Declarations

Ethics Approval and Consent to Participate

This study was performed with the disclosure of information according to the approval of the Clinical Studies Ethics Committee of Nagasaki University Hospital (approval no. 18121007) with opt-out disclosure operation.

Consent for publication

All authors have approved publication of this study.

Availability of data and material

All authors have accepted sharing our data in this manuscript.

Competing interests

The authors declare no conflict of interest.

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We used our department's trust accounts for this study.

Authors' contributions:

Study conception and design: H. Nakamura

Salivary gland biopsy: T. Shimizu, A. Takatani, S. Nishihata

Acquisition of data: H. Nakamura, S. Morimoto

Statistical analysis: S. Morimoto

Interpretation of data: T. Shimizu, A. Takatani, H. Nakamura, A. Kawakami

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Figures

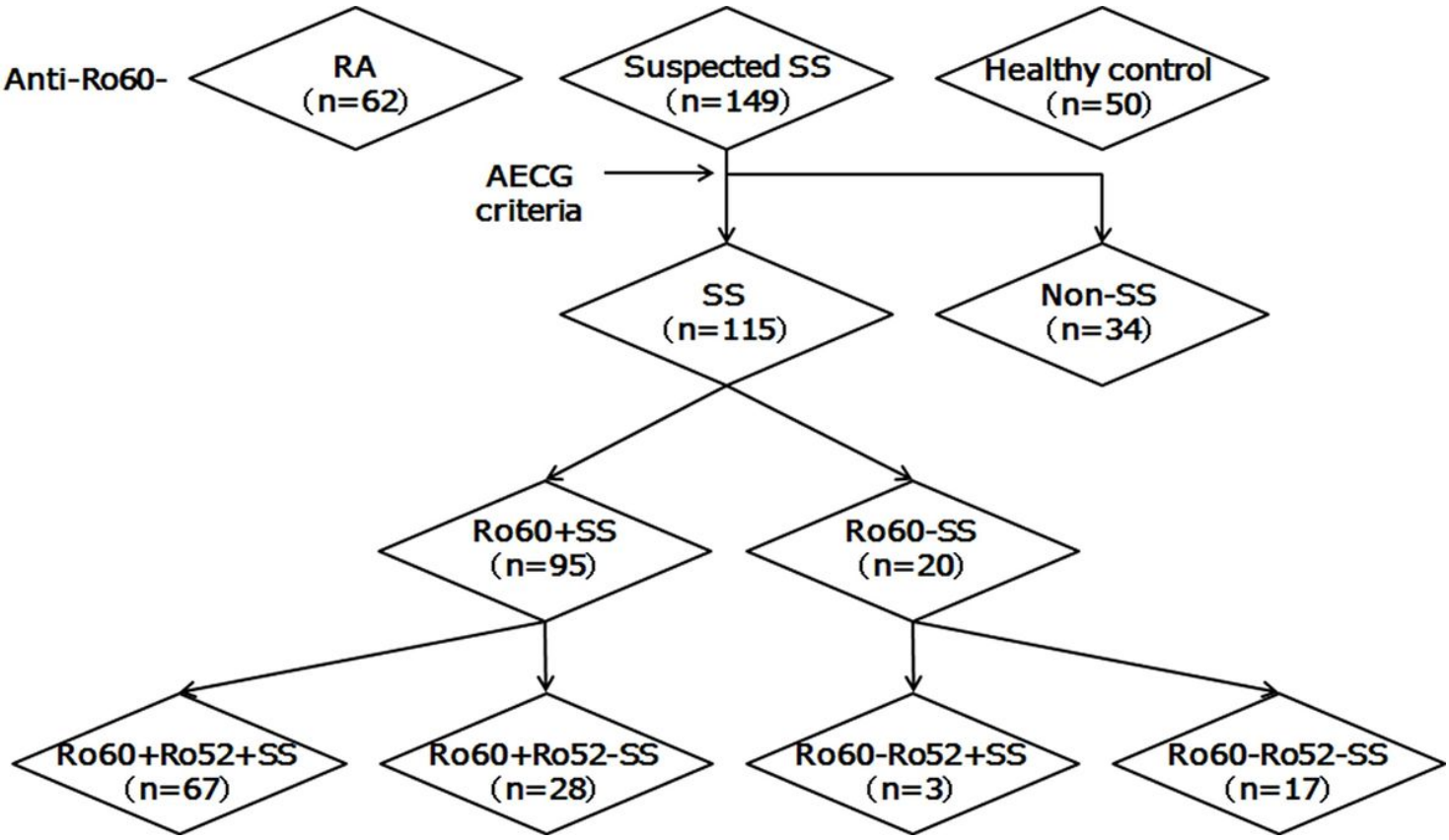

Figure 1

Enrolled patients with SS by anti-Ro positivity. We classified 149 subjects with suspected SS into SS and non-SS groups based on the AECG criteria. The SS group was then divided according to anti-Ro52 and anti-Ro60. The cases of 62 patients with RA and 50 healthy subjects were also analyzed.

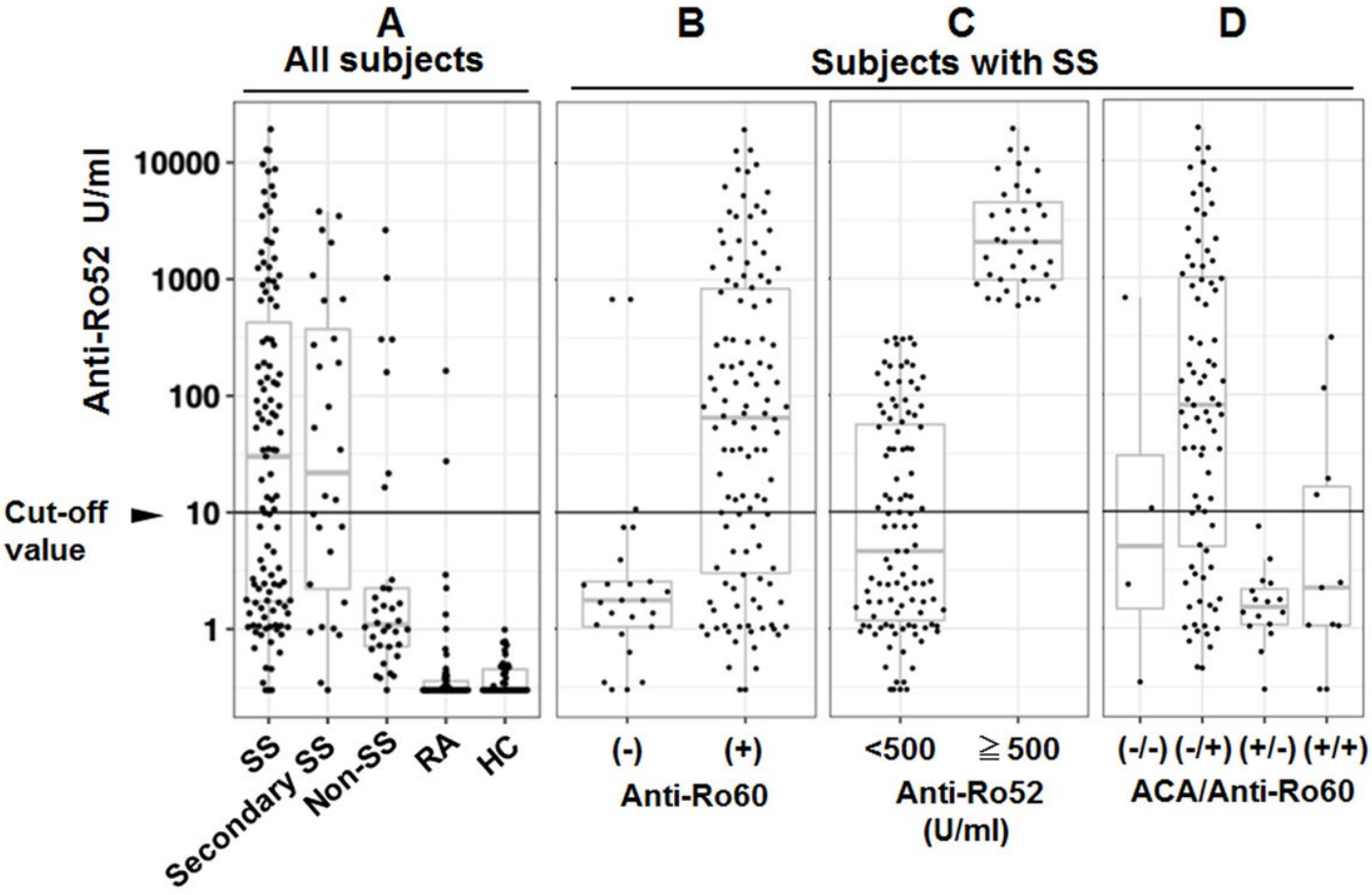

Figure 2

The relevance and distribution of anti-Ro52-seropositive SS patients. The boxes and whiskers in the respective plots represent the interquartile ranges and the ranges of 1.5 times of the 1st and 3rd quartile points. Horizontal lines: the median values. A: All subjects according to the disease group (SS; n=115, secondary SS; n=28, non-SS; n=34, RA; n=62, and healthy subjects; n=50). B,C: SS subjects according to anti-Ro60 seropositivity (B) and markedly elevated (≧500 U/ml) anti-Ro52 concentration titer (C). D: SS subjects with (+)/without (−) ACA seropositivity according to anti-Ro60 seropositivity. ACA: anti-centromere antibody. HC: healthy subjects, RA: rheumatoid arthritis.

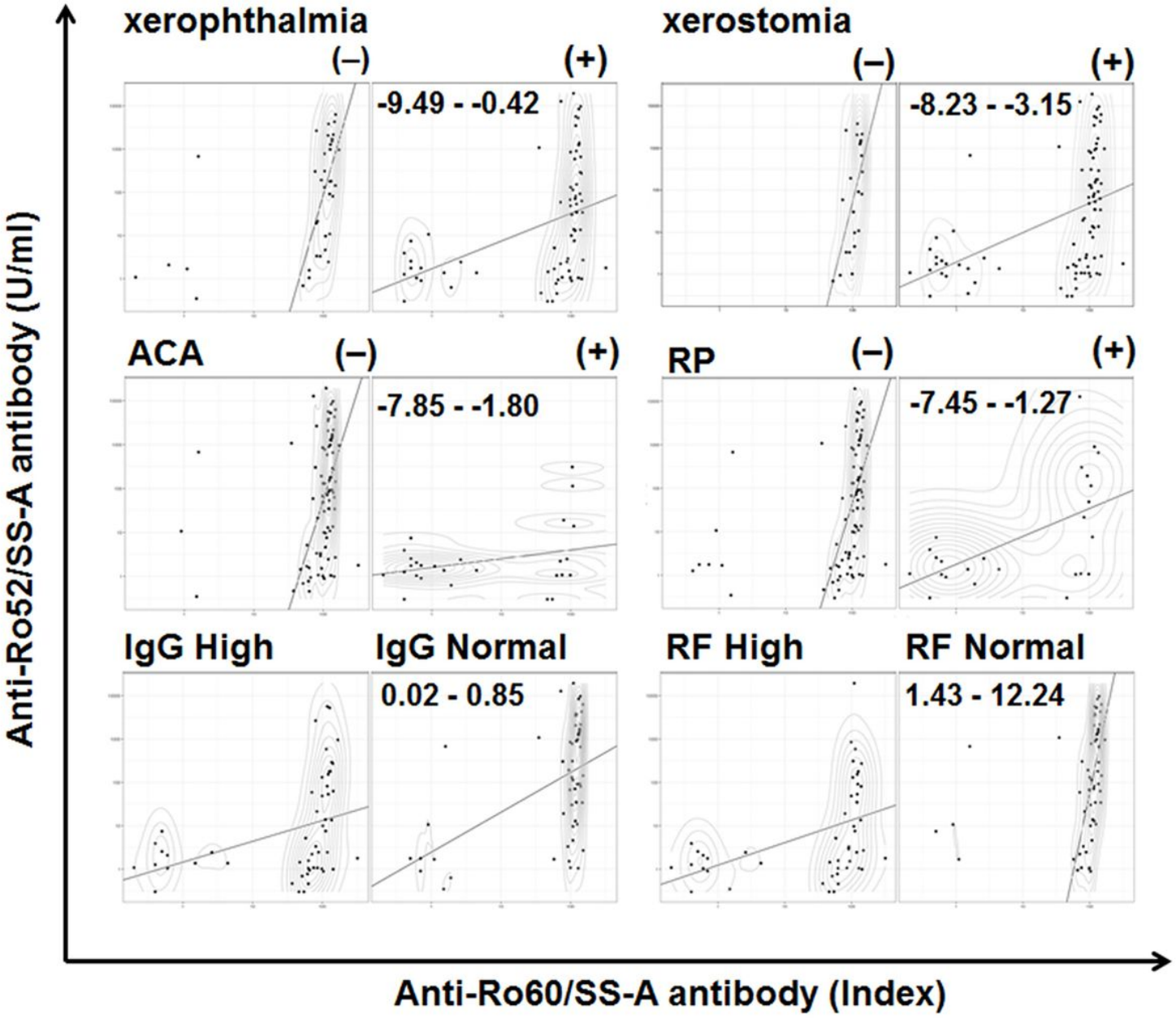

Figure 3

The clinical characteristics relevant to the relationship between anti-Ro52 and anti-Ro60. The six clinical parameters that showed significance in the relevance to relationship between anti-Ro52 and anti-Ro60. The levels of anti-Ro-60 and anti-Ro52 are coordinated on the x- and y-axes with a logarithmic scale, respectively. The regression line from the linear regression model analysis is shown in each panel. The contour in each panel was estimated based on two-dimensional density. ACA: anti-centromere antibody, RF: rheumatoid factor, RP: Raynaud's phenomenon.

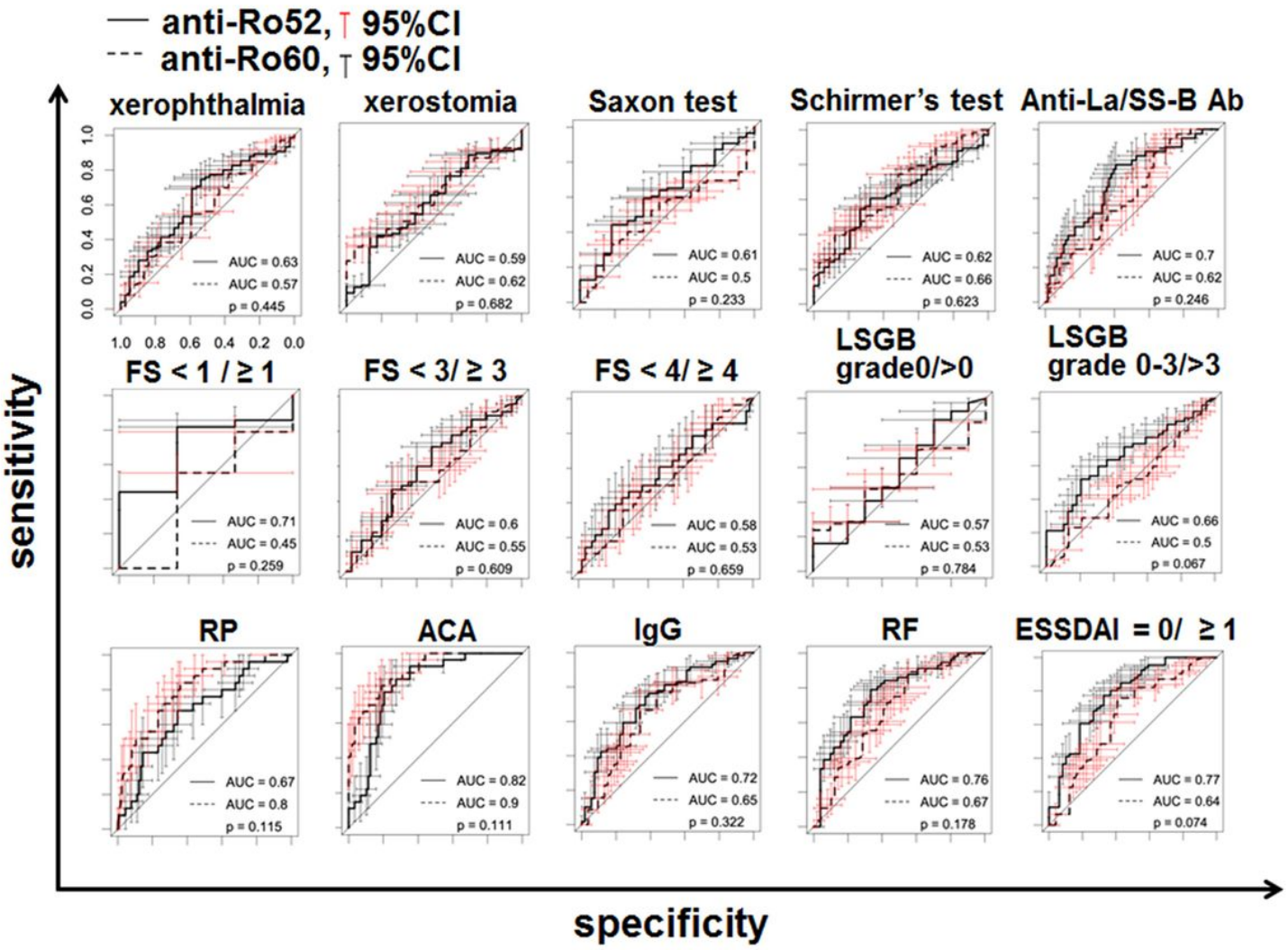

Figure 4

The clinical characteristics relevant to the transition of the anti-Ro52 concentration. The ROC curves of the levels of anti-Ro52 and anti-Ro60 for each clinical characteristic are shown. The clinical characteristics were 10 AECG components and five other factors. The p-value in each panel is the result of a test of the null hypothesis that the AUC of the anti-Ro52 ROC curve was 0.5. Ab: antibody, ACA: anti-centromere antibody, ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index, FS: focus score, LSGB: labial salivary gland biopsy, RF: rheumatoid factor, RP: Raynaud's phenomenon.

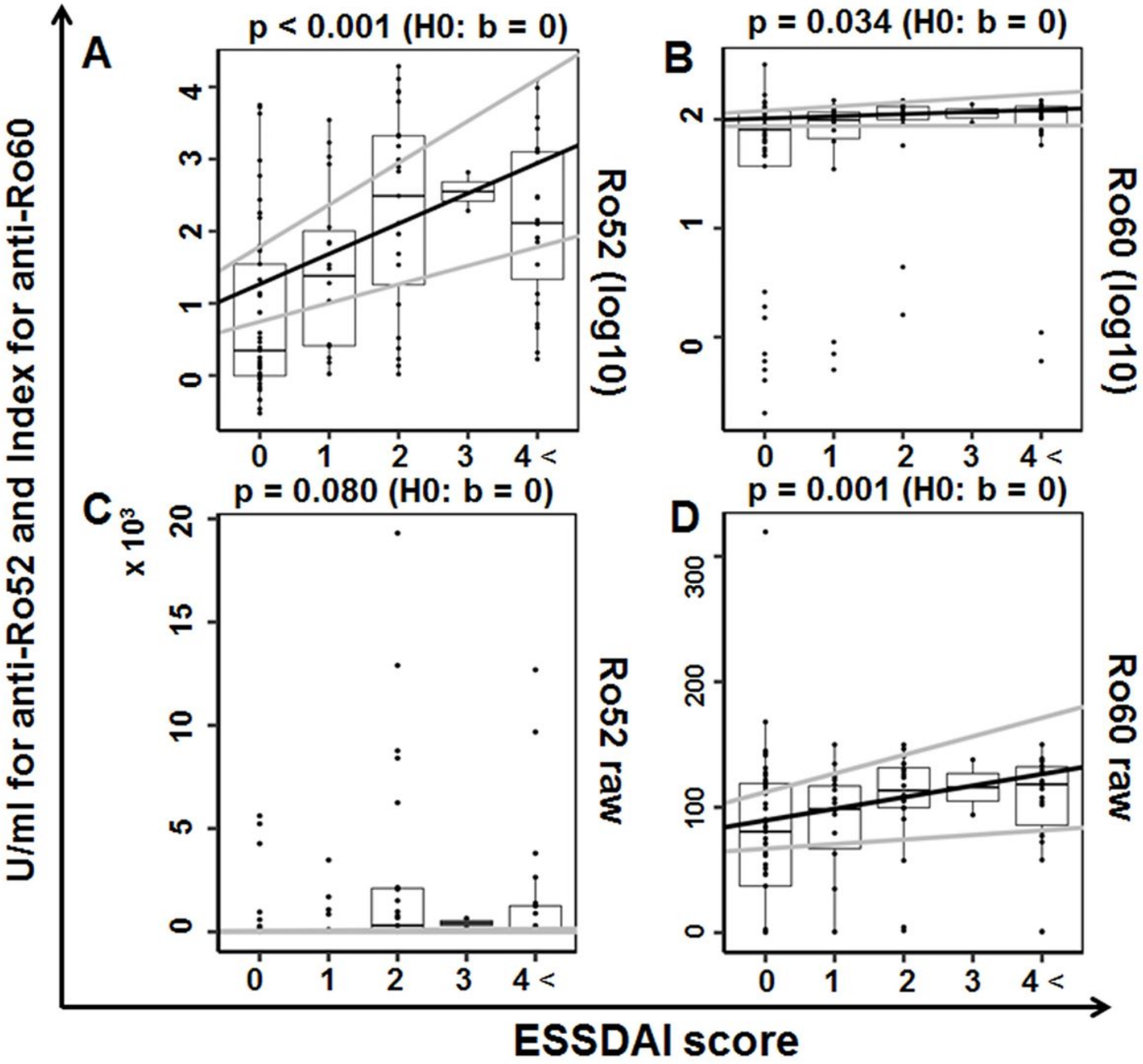

Figure 5

The relevance of anti-Ro52/60 antibodies according to ESSDAI score. The line with the intercept and slope obtained from the linear regression (gray line: 95%CI) and box-whisker plots (boxes: interquartile ranges; whiskers extend from the upper/lower hinges to the highest/lowest value no further than 1.5 times the ranges of the 1st and 3rd quartile points). The y-axis data were scaled on logarithmic with base of 10 in panels A and B and not scaled in panels C and D. ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index.

Supplementary Files

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